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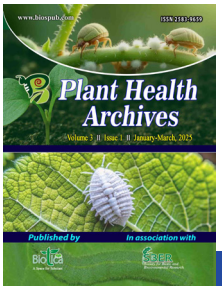

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Research Article

Studies on Cultural and Morphological Variability in the Isolates of *Fusarium solani* Causing Dieback Disease in Tea Ecosystem of NE India

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Abstract

Total 10 *Fusarium solani* were isolated and coded as NMFst1, NMFst2, NMFst3, NMFst4, UMFst1, UMFst2, UMFst3, PAFst, SDFst and CDFst from the tea ecosystem of three North Eastern states of India *viz*., Meghalaya, Assam and Arunachal Pradesh. The isolates were characterized based on cultural characteristics on colony colour (as per Royal Horticultural Society colour chart), zonation (present or absent), topography (raise and umbonate) and sporulation (profuse or moderate) were studied. Morphological characteristics of the isolated *Fusarium solani* grown on PDA medium and microscopic observations were made under 40X. Size of the macro conidia and for the microconidia were found to be in the range of 5.79-23.50×1.29-2.33 µm and 3.12-8.58×1.22-1.88 µm, respectively. Macro conidia are found to be sickle shape (all the isolates) with blunt end (PAFst) and microconidia were round to oval (all isolates) in shape. The colour of mycelium was found to be hyaline. Presence of septation in macroconidia (5-3) and mycelium were also recorded. By comparing the cultural and morphological characteristics with the key guidelines of C. Booth (1971), species level identification was done. Screening for the first growing isolate of *Fusarium solani* was done by taking the radial growth of the isolates at different days after incubation (up to 168 hours). Among the 10 isolates, 6 isolates were found to be fast growing in nature.

Keywords: Cultural characteristics, Dieback disease, *Fusarium solani*, Morphological characteristics, Tea

Introduction

Fusarium solani is considered as one of most prevalent fungal pathogens that can cause diseases more than 100 numbers of agricultural crops. That pathogen can cause vascular wilt, root rot, crown rot as well as dieback disease. The pathogen can survive in the soil by forming thick walled spores like chlamydospores, which can withstand adverse climatic conditions, nutrient depletion and can also persist under chemical stress conditions (Dutta *et al*., 2020; Dutta *et al*., 2022). Several studies have revealed that *F. solani* can show considerable variability in the cultural and morphological characteristics and also shows pathogenic variability during infection processes in plants. This pathogen is considered as one of the destructive foliar fungal pathogens in plantation crops like Tea (*Camellia sinensis* L.) which can cause yield losses up to 20-60% under field conditions (Pandey *et al*., 2024). But under favourable climatic conditions, this pathogen can cause complete yield losses (Babu *et al*., 2022). In India, this pathogen was initially reported to cause disease during the month of June-September. But recent studies have revealed that, this pathogen can cause disease in the tea crop throughout the year by affecting the pluckable shoots of the tea crop leading to dieback disease (Pandey *et al*., 2023). *F. solani* can also impart variation in their cultural and morphological characteristic like colony colour, pigmentation, growth rate *etc*. when grown on cultural medium (Gogoi *et al*., 2017). Due to variation in the isolates of *Fusarium solani*, it is important to know which isolate is the most virulent within a given spices of *Fusarium solani* species complex (Chandran and Kumar, 2012; Debnath *et al*., 2023). In this present study, several isolates of *Fusarium*

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solani were isolated from the tea gardens of three states of NE India *viz*. Meghalaya, Assam and Arunachal Pradesh and their cultural and morphological variabilities were studied.

Materials and Methods

Survey and Collection of Dieback Infested Tea Twig Samples

Survey and collection of dieback infested samples were done at the three gardens from the three states of North East India *viz*. Meghalaya, Assam and Arunachal Pradesh. The twig samples showing prominent symptoms of dieback were collected and putative pathogen was isolated.

Isolation and Purification of Pathogen Causing Dieback Disease in Tea

For the isolation of putative pathogen causing dieback from the infected tea twig samples collected from the selected gardens of each state (Meghalaya, Assam and Arunachal Pradesh) were first washed under running tap water to remove all visible soil particles. Freshly infected samples were selected for the isolation of the putative pathogen. The infected tissues were cut into small pieces 3-5 mm long and surface sterilized in 4% sodium hypochlorite solution for 1 to 2 min followed by rinsed in 70% ethanol and then it was washed twice in sterile distilled water to remove the sterilizing agent. Samples were then dried in sterile filter papers. Surface disinfected pieces were placed on potato dextrose agar (PDA) medium accompanied with 20 ppm of streptomycin sulphate to avoid contamination. Plates were then incubated at 28±2 °C for 6-7 days till full plate growth of the pathogen occurred in the inoculated plates. Purification of the targeted pathogen was done by following hyphal tip culture method (Choi *et al*., 1999). The pure cultures were transferred to PDA slants which were maintained by regular sub culturing at an interval of 2 months and kept at 4±1 °C in refrigerator.

Pathogenicity Test

For the pathogenicity of the *Fusarium solani* causing dieback diseases in Tea, healthy seedlings collected from the Mirem Tea Garden (MTG), East Siang, Arunachal Pradesh were selected. Pathogenicity test was done by spraying the fungal spore suspension on the root zone of the tea seedlings as described by Devi *et al*. (2012). The spore suspension of the twig dieback pathogen of tea was prepared from 7 days old actively growing culture on PDA medium that was swirled and scraped in sterile distilled water. The spore suspension was adjusted to 1×10^6 spore ml^{-1} using a Haemocytometer and was drenched onto the root zone of the young tea seedlings under pot condition. Spore suspension was injected to the stems while sterile distilled water sprayed in control treatment. The saplings were then covered with perforated polythene to avoid secondary contamination and maintain humidity for 72 hrs and observation of the dieback symptoms were made. The pots were observed for 28 days after inoculation and development of characteristics symptoms were observed and pathogen was re-isolated from the infected twig and compared with the original culture to prove the pathogenicity test (Figure 1).

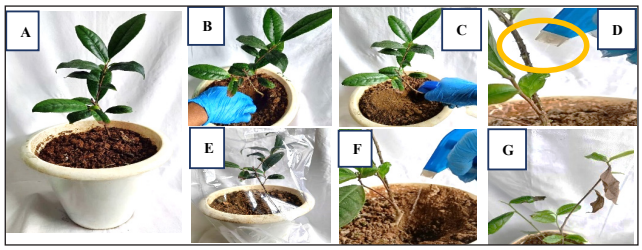


Figure 1: Pathogenicity test for dieback disease in tea [A: Healthy tea seedling; B, C and D: Application of fungal spore suspension (1×10^6 cfu ml^{-1}) in root and injured stem; E: Saplings covered with perforated polythene to maintain humidity; F (Control): Application of sterile distilled water in the uninoculated control; G: Dieback symptom in infected tea seedling]

Cultural and Morphological Characterisation

The cultural characteristics of all the isolates of the pathogen causing dieback diseases in tea were studied on potato dextrose agar (PDA) medium. Mycelial disc (5 mm) of young growing cultures of all the isolates of the pathogen were inoculated (centrally) in the Petri plates containing sterilized solidified media and incubated at 28±2 °C for one week. Three plates were taken from each isolates and cultural characteristics were recorded on colony colour, zonation, topography and growth rate. Morphological identification of all the isolates were studied on characters *viz*., size of the conidia, septation, shape of the conidia and colour (Rezaee *et al*., 2018). Microscopic examination was carried out at 40X magnification by mounting the culture in lactophenol cotton blue (Agu and Chidozie, 2021).

Screening for Fast Growing Isolates of Fusarium solani

Screenings of fast-growing *Fusarium* isolates were done by measuring the radial growth of the pathogen (in mm) on daily basis up to 168 hrs (7 days). The isolates of *Fusarium solani* were inoculated in Potato Dextrose Agar (PDA) media having pH 5.6±0.2 and plates were incubated at 28±2 °C. Among the isolates, the first growing isolates were scored with (***) mark, moderately growing isolates were scored with (**) mark and slow growing isolates were marked with (*) respectively.

Results and Discussion

Total ten (10) *Fusarium solani* were isolated from the collated diseased samples and purified by the pure hyphal tip culture method. Details of collection sites with locations and geographical coordinates were recorded and listed in table 1. Out of the three states sample, seven (7) *Fusarium solani* were isolated from Meghalaya, among which four (4) isolates were from Nalapara Tea Garden (NTG), Ri-Bhoi, Meghalaya, three (3) isolates from Tea Development Centre (TDC), Directorate of Horticulture (DOH), Umsning, Ri-Bhoi. In Assam, one (1) *Fusarium solani* was isolated from Saslapara New Tea Garden (Saslapara NTG) and one (1) isolate from Maa Phulaswari Mini Tea Garden (Maa Phulaswari MTG), but no isolates were isolated from Experimental Garden for Plantation Crops (EGPC), Jorhat, as there was no record of dieback infestation in the later. From Arunachal Pradesh,

Table 1: Details of collection sites with isolate code, location and geographical coordinates for the *Fusarium solani* isolates from the tea ecosystem of Meghalaya, Assam and Arunachal Pradesh

State	Isolate code*	Name of the garden	GPS Coordinates	
			Latitude	Longitude
Meghalaya	NMFst1, NMFst2, NMFst3, NMFst4	Nalapara Tea Garden (NTG), Ri-Bhoi	25.7322° N	91.8832° E
	UMFst1, UMFst2, UMFst3	Tea Development Centre (TDC), Directorate of Horticulture (DOH), Umsning, Ri-Bhoi	25.7311° N	91.8874° E
	No isolates	Andersan Tea Estate (ATE), Ri-Bhoi	25.8714° N	91.8280° E
Assam	SDFst	Saslapara New Tea Garden (Saslapara NTG), Dhubri	26.3688° N	90.4126° E
	CDFst	MaaPhulaswari Minin Tea Garden (MaaPhulaswari MTG), Hindupara, Dhubri	26.3278° N	90.4208° E
	No isolates	Experimental Garden for Plantation Crops (EGPC), Jorhat	26.7178° N	94.1988° E
Arunachal Pradesh	PAFst	Pao Tea Garden (PTG), East Siang	27.8562° N	95.2532° E
	No isolates	Merim Tea Garden (MTG), East Siang	27.9588° N	95.3406° E
	No isolates	Lingka Tea Garden (LTG), East Siang	27.8756° N	95.2532° E

out of three (3) gardens, only one (1) *Fusarium solani* isolate was isolated from the samples collected from Pao Tea Garden (PTG). But from Lingka Tea Garden (LTG) and Merim Tea Garden (MTG) of Arunachal Pradesh, no dieback infestation was observed during survey and collection of diseased samples.

Cultural and Morphological Characteristics of the Isolates of Fusarium solani

Cultural characterisation pertaining to colony colour, zonation, topography and sporulation were studied for ten (10) *Fusarium solani* isolates causing die back disease in tea, whose samples were collected from tea gardens of Meghalaya, Assam and Arunachal Pradesh. The isolates were grown on PDA medium and record on the cultural characteristics, were taken after the 7th day of incubation. Data presented in table 2 showed the details of cultural characteristics of *Fusarium solani*. For the determination of colony colour, front and reverse view of the cultures were recorded by following the Royal Horticultural Colour Chart (R.H.S. Colour Chart) (Figure 2). Among the ten (10) *Fusarium solani* isolates, four (4) isolates showed orange-white group (UMFst2, UMFst3, CDFst and SDFst). Three (3) isolates (NMFst1, NMFst3 and UMFst1) showed profuse sporulation, five (5) isolates (NMFst2, NMFst4, UMFst2, UMFst3 and PAFst) showed moderate sporulation and remaining two (2) isolates (CDFst and SDFst) showed slow sporulation (Table 2). Profuse to moderate type of sporulation were recorded in all the isolates which was found in the line with the cultural and morphological variability of *Fusarium solani* studied earlier by Chandran and Kumar (2012). Among the ten (10) isolates, zonation was observed in four (4) isolates (NMFst1, NMFst3, UMFst1 and UMFst3) and in remaining six (6) isolates (NMFst2, NMFst3, NMFst4, UMFst2, CDFst, SDFst and PAFst) zonation was absent. During the observation for the topography formed by the isolates in their cultural plates, it was found that, among the ten (10)

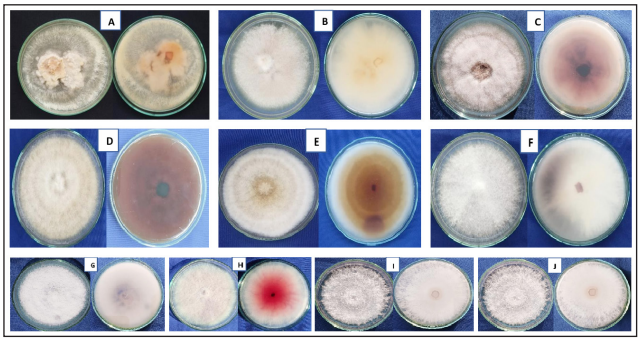


Figure 2: Front and reverse view of isolated *Fusarium solani* from the dieback infested tea samples collected from Meghalaya (A-G), Arunachal Pradesh (H) and Assam (I-J) [A: NMFst1, B: NMFst2, C: NMFst3, D: NMFst4, E: UMFst1, F: UMFst1, G: UMFst3, H: PAFst, I: CDFst, J: SDFst]

isolates, three (3) isolates (NMFst1, NMFst3 and UMFst3) showed raised and umbonate type of topography, three (3) isolates (NMFst2, NMFst4 and UMFst2) showed flat type of topography, three (3) isolates (CDFst, SDFst and PAFst) showed cottony and flat type topography and one (1) isolate (UMFst1) showed spreading thread type of topography in their respective cultural plates after the seven (7) days of incubation (Table 2; Figure 3). Morphological observations (microscopic observations) pertaining to colour, number of septation, size of the conidia and shape of the conidia were studied for ten (10) isolates of *Fusarium solani* isolated from the dieback infested tea twigs collected from the tea gardens of Meghalaya, Assam and Arunachal Pradesh (Table 3). The morphological observation was recorded after the 7th day of incubation of cultures grown on PDA medium. The hyphae of *Fusarium solani* isolates were observed as septate and hyaline in colour. The macro-conidia were mostly found to be sickle shape with (3-5) septa and microconidia were found to be round to oval in shaped without septation. Chlamydospores are also formed both terminally and intercalary and phialides are hyaline and branched in nature.

Table 2: Cultural characteristics of *Fusarium solani* isolates on PDA medium after 7th days post incubation

State	Isolates	Colony Colour*		Zonation	Topography	Sporulation
		Front view	Reverse view			
Meghalaya	NMFst1	Grey-yellow group B 16 (-)	Greyed-orange group A 163 (Bronze yellow)	Present	Raised and Umbonate	Profuse
	NMFst2	Yellow-white group B 158 (-)	Greyed-yellow group B 161 (-)	Absent	Flat	Moderate
	NMFst3	Orange group D 28 (-)	Greyed-orange group B 165 (Almond shell BCC 67)	Present	Raised and Umbonate	Profuse
	NMFst4	Greyed-orange group C 163 (-)	Greyed-orange group A 167 (-)	Absent	Flat	Moderate
	UMFst1	Greyed-orange group B 164 (Golden buff CC221)	Greyed-orange group C 166 (Squirrel brown CC 208)	Present	Spreading thread type	Profuse
	UMFst2	Orange-white group A 159 (-)	Yellow-white group A 158 (Chrome yellow HCC 6053)	Absent	Flat	Moderate
	UMFst3	Orange-white group A 159 (Honey suckle BCC62)	Yellow-white group B 158 (-)	Present	Raised and umbonate	Moderate
Assam	CDFst	Orange-white group D (-)	Greyed-yellow group B 161 (-)	Absent	Cottony and flat	Slow
	SDFst	Orange-white group D (-)	Greyed-yellow group B 161 (-)	Absent	Cottony and flat	Slow
Arunachal Pradesh	PAFst	Greyed-orange group C 62 (orchid pink BCC 106)	Greyed-Orange group A 177 (-)	Absent	Cottony and flat	Moderate

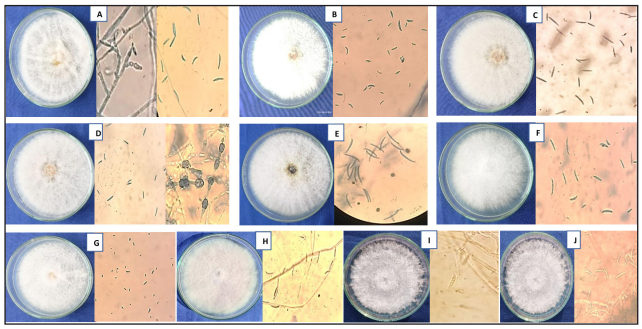


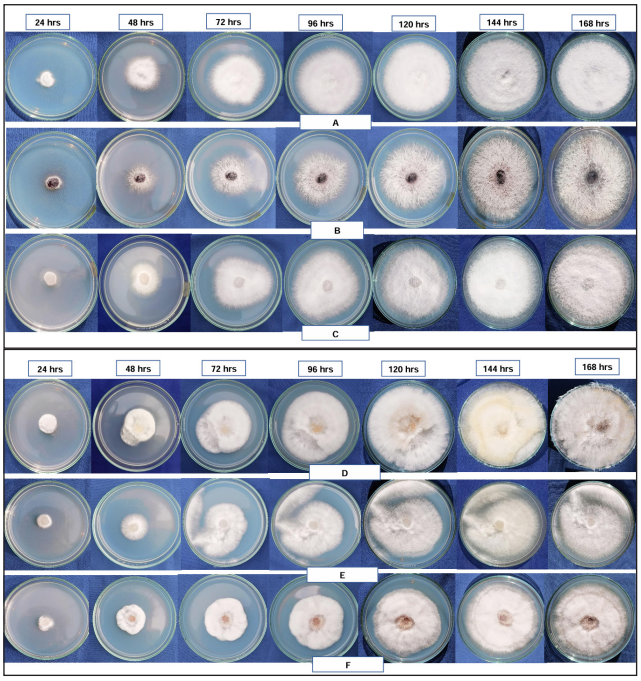
Figure 3: Microscopic variability of *Fusarium solani* isolates from tea gardens of Meghalaya [A: NMFst1; B: NMFst2; C: NMFst3; D: NMFst4; E: UMFst1; F: UMFst2; G: UMFst3; H: Arunachal Pradesh; I-J: PAFst and Assam (I: CDFst, J: SDFst); Microscopic observation (40X)]

For the size of the macro conidia and for the microconidia was found to be in the range of 5.79-23.50×1.29-2.33 µm and 3.12-8.58×1.22-1.88 µm, respectively. Similar records on morphological characteristics were also reported by Chandran and Kumar (2012) and Chetri *et al*. (2015).

For the species level identification of *Fusarium solani*, recorded data on cultural and morphological characteristics were compared with literature published by C. Booth (1971) and after comparing the isolates were identified as *Fusarium solani*.

Screening for Fast Growing Fusarium solani Isolates

Among the ten (10) isolates of *F. solani*, the most fast-growing isolates of the *F. solani* was screened for which the radial growth was taken at an interval of 24 hrs to 168 hrs (7 days) of post incubation on PDA medium (Table 4; Figure 4). The isolates were inoculated in the Petri plates containing PDA medium of pH 5.6±0.2. The plates were then incubated at temperature 28±2 °C. The rating for the fast-growing *F. solani* was done with a scale of *: <69 mm (slow growing),



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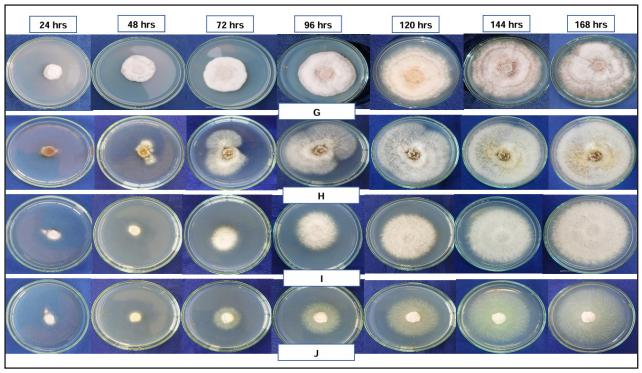

Figure 4: Radial growth of *Fusarium solani* grown on PDA medium up to 168 hrs after the inoculation [A: UMFst3; B: PAFst; C: UMFst2; D: NMFst1; E: NMFst2; E: NMFst3; F: NMFSt3; G: NMFst4; H: UMFst1; I: CDFst and J: SDFst]

: 70-75 mm (moderately growing) and *: >73 mm (fast growing). Among the ten (10) isolates, six (6) isolates were found to have fast radial growth rate *viz*., NMFst1, UMFst1 and UMFst3 (75mm), NMFst2 (74.3 mm), UMFst2 (73.6 mm) and PAFst (73.7 mm). Two (2) isolates *viz*., NMFst3 (72.0 mm) and NMFst4 (72.6 mm) have showed moderate growth rate and the two (2) isolates CDFst and SDFSt of gardens of Dhubri district showed slow growth rate with 69.6 mm and 68.2 mm, respectively after the 7th day of incubation (Figure 5). Raghu *et al*. (2016) reported higher degree of variability among the isolates of *Fusarium* spp. in terms of growth rate and highlighted the importance of molecular level of identification to study up to the genomic level of the pathogen. So, it can be said that growth rate or pattern of different isolates of an organism may vary which may be genetical characteristics governed by some major gene that responsible for fastness of their growth and differences in the nutrient requirement of the isolates (Brasileiro *et al*., 2004; Arif *et al*., 2008; Zaccardelli *et al*., 2008).

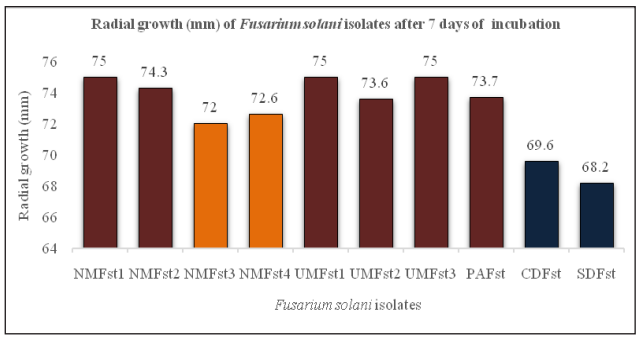

Figure 5: Radial growth (mm) of *Fusarium solani* isolates from the dieback infested samples collected from the tea ecosystem of Meghalaya, Assam and Arunachal Pradesh after 7 days of incubation

Table 3: Morphological characteristics of isolates of *Fusarium solani* pertaining to shape, colour and size of conidia grown on PDA medium

State	Isolate	Size of conidia (µm)*		Septation		Shape of conidia		Colour
		Macro conidia (LXB)	Micro conidia (LXB)	Macro conidia (No.)	Micro conidia (No.)	Macro conidia	Micro conidia	
Meghalaya	NMFst1	23.50-(13.80)-8.26X 2.19-(1.97)-1.28	8.58-(7.01)-6.67X1.88-(1.63)-1.26	4-5	0-1	Sickle	Round to oval	Hyaline
	NMFst2	12.12-(8.43)-5.79X 2.13-(1.56)-1.24	8.02-(6.23)-5.34X1.78-(1.58)-1.29	3-5	0-1	Sickle	Round to oval	Hyaline
	NMFst3	12.62-(9.71)-8.34X2.12-(1.82)-1.31	8.32-(6.83)-5.21X1.72-(1.44)-1.43	4-5	0-1	Sickle	Round to oval	Hyaline
	NMFst4	16.81-(13.36)-10.42X2.22-(1.43)-1.21	7.16-(6.52)-5.22X1.65-(1.43)-1.21	3-5	0-1	Sickle	Round to oval	Hyaline
	UMFst1	20.56-(17.74)-12.20X2.24-(1.86)-1.28	8.25-(7.11)-6.32X1.62-(1.60)-1.28	3-6	0-1	Sickle	Round to oval	Hyaline
	UMFst2	12.36-(7.32)-5.83X2.33-(1.77)-1.24	6.33-(4.23)-3.16X1.52-(1.46)-1.25	3-4	0-1	Sickle	Round to oval	Hyaline
	UMFst3	12.13-(8.34)-5.88X2.17-(1.64)-1.23	5.62-(4.83)-3.21X1.61-(1.58)-1.32	3-5	0-1	Sickle	Round to oval	Hyaline
Assam	CDFst	15.26-(12.13)-8.44X2.15-(1.53)-1.29	5.21-(4.13)-3.34X1.55-(1.43)-1.26	3-5	0-1	Sickle	Round to oval	Hyaline
	SDFst	16.26-(12.18)-8.55X2.16-(1.43)-1.21	6.22-(4.21)-3.12X1.63-(1.43)-1.26	3-5	0-1	Sickle	Round to oval	Hyaline
Arunachal Pradesh	PAFst	15.59-(12.85)-8.73X2.15-(1.43)-1.23	6.05-(4.25)-3.11X1.61-(1.44)-1.22	3-5	0-1	Sickle shape with blunt end	Round to oval	Hyaline

Table 4: Growth rate of *Fusarium solani* isolates from Meghalaya, Assam and Arunachal Pradesh at different days of incubation (24 hrs - 168 hrs)

State	Isolates	Radial growth of *Fusarium solani* (mm)							
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	Score
Meghalaya	NMFst1	16.6	29.6	47.2	57.4	57.4	75.0	75.0	***
	NMFst2	14.6	24.2	42.6	52.0	52.0	69.7	74.3	***
	NMFst3	13.6	26.6	38.0	46.2	46.2	63.2	72.0	**
	NMFst4	13.8	28.6	36.2	46.6	46.6	69.6	72.6	**
	UMFst1	11.6	22.8	42.6	59.2	59.2	73.7	75.0	***
	UMFst2	17.4	28.6	49.8	58.4	58.4	70.6	73.6	***
	UMFst3	14.2	29.6	42.4	59.2	59.2	70.4	75.0	***
Assam	CDFst	12.2	17.4	27.8	38.4	38.4	58.8	69.6	*
	SDFst	12.5	17.6	23.4	36.0	36.0	55.6	68.2	*
Arunachal Pradesh	PAFst	11.0	24.6	36.0	49.6	49.6	63.4	73.7	***

***: Fast growing, **: Moderately growing, *: Slow growing

Conclusion

In present study, *Fusarium solani* causing dieback diseases in tea were isolated and cultural and morphological variabilities among the isolates were recorded. Further, for species level identification, the cultural and morphological characteristics were compared with the key guidelines given by C. Booth (1971). Screening for fast growing isolates of *F. solani* was done by taking the radial growth of the isolates grown on PDA medium up to 7 days and variation in the rate of growth among the isolates were observed. Differences in the cultural and morphological characteristics among the isolates of *F. solani* may be due to variation in the geographical and environmental conditions. In future, combined studies on molecular and classical approaches should be made to understand these variabilities among the isolates of *F. solani* causing dieback disease in tea ecosystem.

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